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Linking Local Circuit Inhibition to Olfactory Behavior: A Critical Role for Granule Cells in Olfactory Discrimination

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In this issue of *Neuron*, Abraham et al. report a direct connection between inhibitory function and olfactory behavior. Using molecular methods to alter glutamate receptor subunit composition in olfactory bulb granule cells, the authors found a selective modulation in the time required for difficult, but not simple, olfactory discrimination tasks.

The standard depiction of our sense of smell emphasizes the serial processing of sensory information by three olfactory brain regions. Chemical stimuli are transduced into spike train patterns by receptor neurons in the nasal epithelium. Action potentials in receptor cell axons release glutamate onto distal dendrites of mitral and tufted cells, activating these principal cell types in the olfactory bulb. Tertiary olfactory regions, such as piriform cortex and the olfactory tubercle, receive excitatory input from mitral and tufted cells and presumably from associations between olfactory bulb output patterns and stimuli in the environment.

This simplistic description ignores two prominent, but understudied, features of the olfactory system: the role local inhibitory interneurons play in the olfactory bulb and the massive feedback projections from cortical regions that preferentially target bulbar interneurons (Shepherd and Greer, 1998). Two decades ago, pioneering in vivo intracellular recordings from salamander mitral cells by Hamilton and Kauer (1989) directly demonstrated that large-amplitude inhibitory responses dramatically shape the odor-driven output of the olfactory bulb. Presumably, much of this inhibition arises from granule cells, the most numerous GABAergic interneuron subtype in the olfactory bulb (Shepherd and Greer, 1998). Unlike most CNS regions, inhibitory interneurons far outnumber principal cells, by at least 50:1, in the olfactory bulb. Despite intense efforts to understand the synaptic circuits that mediate this inhibition in vitro (Isaacson and Strowbridge, 1998; Isaacson, 2001; Chen et al., 2000; Schoppa and Westbrook, 1999), we know relatively little about how granule cells function during olfaction or why some mitral cell responses are dominated by inhibition while other responses appear to reflect mostly excitation from receptor cells.

In a report in this issue of *Neuron*, Abraham et al. (2010) use a powerful combination of molecular biological techniques to directly modulate granule cell function in mice in vivo. Using this innovative method, they link a specific aspect of olfaction (discrimination latency) to granule cell-mediated inhibition. Their study also extends into mammals a critical distinction between "easy" and "difficult" olfactory sensory tasks that arose from related work on insects (Stopfer et al., 1997). Both mammals and insects appear to be able to differentiate between very different stimuli using only excitatory connections between principal cells in olfactory brain regions. However, inhibitory local circuits in second-order brain regions, such as the olfactory bulb, are required for good performance on difficult tasks, such as discriminating between mixtures of two odorants at different concentration ratios. In musical terms, local inhibition appears to make the difference between playing the piano one key at a time instead of with all ten fingers simultaneously. The former is crude but effective in conveying information as long as the melody is simple.

Assessing interneuron function in the olfactory bulb has been notoriously difficult. As one can guess from their name, granule cell bodies are small and, therefore, difficult to record from using extracellular or intracellular methods. Granule cells also lack an axon. Instead of forming conventional presynaptic terminals, they release neurotransmitter from specialized large dendritic spines that form reciprocal synapses with lateral dendrites of mitral and tufted cells (Rall et al., 1966). Through these dendrodendritic microcircuits, glutamate released from a mitral or tufted cell dendrite can excite granule cell spines, triggering recurrent inhibition back onto the principal cell ("self-inhibition"; Jahr and Nicoll, 1980). The same microcircuit also can mediate lateral inhibition onto other mitral or tufted cells if the depolarization spreads through the granule cell dendritic tree, liberating GABA from other spines (Isaacson and Strowbridge, 1998).

To selectively manipulate reciprocal dendrodendritic synapses, Abraham and colleagues altered the normal complement of glutamate receptors granule cells express, modestly increasing or decreasing excitatory drive to these interneurons. The most robust findings came from increasing excitation of granule cells by eliminating the ionotropic glutamate receptor subunit GluA2 that functions to inhibit Ca^{2+} entry through AMPA receptors. GluA2 expression was blocked by stereotaxically injecting an adenovirus encoding Cre recombinase directly into the granule cell layer of transgenic mice carrying conditional GluA2 alleles. Changing AMPA receptors in excitatory synapses on about half of the population of granule cells from a mixture of GluA1 and GluA2 subunits to primarily GluA1

subunits would be expected to have an especially dramatic effect since many of these receptors reside on dendritic spines that release GABA through Ca^{2+}-dependent mechanisms (Isaacson, 2001; Isaacson and Strowbridge, 1998). By boosting Ca^{2+} influx through AMPA receptors, excitatory inputs to granule cells should trigger more GABA release and thereby generate more inhibition onto mitral and tufted cells. Another advantage is that this manipulation conveys some selectivity to dendrodendritic synapses since only these granule cell spines are both pre- and postsynaptic; most other excitatory inputs to granule cells terminate on solely postsynaptic spines along proximal dendrites. At these synapses, increasing Ca^{2+} influx through AMPA receptors cannot trigger GABA exocytosis directly. There will likely still be some enhancement of cortical feedback to granule cells, the source of most proximal input (Balu et al., 2007), following virus infection reflecting the more depolarized driving force for AMPA receptors. However, this effect should be smaller than at dendrodendritic synapses where the increased Ca^{2+} influx will likely have both immediate electrical and chemical signaling effects.

Abraham et al. demonstrated enhanced Ca^{2+} accumulations in granule cell spines following electrical stimulation in acute olfactory bulb slices prepared from virus-infected mice. Self-inhibition of mitral cells following trains of action potentials also was significantly increased in both in vitro and in vivo recording conditions. There were no apparent compensatory changes in GluA1 subunits or obvious changes in granule cell morphology.

The payoff from this complicated molecular modulation strategy was the surprising finding that modestly enhancing dendrodendritic inhibition improves olfactory function. There was no effect when mice were trained to discriminate between two very different odorants. However, the time mice took to discriminate between two similar stimuli (binary odorant mixtures with different concentration ratios) decreased significantly when AMPA receptors on GABAergic granule cells were made Ca^{2+} permeable. The effect was relatively modest in absolute terms—virus-infected mice discriminated binary mixtures ~50 ms faster than control mice—but is substantial in terms of the range of times rodents generally take to perform this type of task. While much depends on the specific details of the behavioral paradigm used, rodents often take an additional 100 ms to discriminate between two similar stimuli, compared with the time required for an "easy" discrimination task involving two different monomolecular odors (Abraham et al., 2010). Viewed in this context, decreasing discrimination time by 50 ms represents a large functional effect.

Abraham et al. also explored the behavioral effects of decreasing NMDA receptor function by applying the same method to transgenic mice containing conditional GluN1 alleles. This treatment also would be expected to have a relatively large impact on dendrodendritic synapses in the olfactory bulb since GABA release from granule cell dendrites can be driven directly by local NMDA receptors (Halabisky et al., 2000; Chen et al., 2000). While the effect of manipulating NMDA receptors on inhibitory responses was not as large as with GluA2 manipulations, mitral cell inhibition tended to be reduced following infection, and the time required for difficult olfactory discrimination tasks was significantly increased.

Together, these results suggest that granule cell-mediated dendrodendritic inhibition plays an important role during olfactory discrimination tasks. Exactly how inhibition facilitates olfactory discrimination is not clear yet. Dendrodendritic inhibition mediated by granule cells may enable the discharge patterns of mitral and tufted cells, the output of the olfactory bulb, to diverge from the input patterns imposed by receptor cells. This divergence in input/output relationships may be especially important when attempting to distinguish between similar sensory stimuli. Without normal interneuron function, the olfactory bulb may behave more like a simple relay structure, passing olfactory receptor input patterns on to piriform cortex with relatively little processing. As dendrodendritic inhibition increases, the olfactory bulb may generate more distinctive output patterns in response to similar odors—sensory stimuli that presumably generate highly overlapping bulbar input patterns that would be difficult for downstream brain regions to differentiate if they were passed on unprocessed.

Interestingly, neither manipulation affected discrimination accuracy, even during difficult discrimination tasks. Here, the results in mammals differ from the classic study by Stopfer and colleagues (Stopfer et al., 1997), who found that modulating inhibitory function in insects by pharmacological blockade of $GABA_A$ receptors in the second-order olfactory brain structure impaired discrimination accuracy for pairs of similar odors but not for pairs of dissimilar odors. In insects, this manipulation disrupts the population γ-band oscillation normally generated in this brain region during olfactory stimulation. Without this network oscillation to "keep time," downstream neurons may be unable to detect specific sequences of output patterns generated by principal neurons. Mammals also generate large-amplitude γ-band oscillations in their olfactory bulbs in response to sensory stimulation (Bressler and Freeman, 1980), which may be mediated, at least in part, by granule cells (Nusser et al., 2001). One exciting possible future application of these molecular methods is to test in vivo whether granule cells underlie γ-band oscillations in mammals and define the relationship between network oscillations and olfactory function.

If a modest increase in inhibitory circuit activity improves olfactory function, what about big changes? Unfortunately, pushing this strategy further is unlikely to yield even larger improvements. Modestly enhancing principal cell inhibition may help differentiate bulbar output patterns, but presumably at the cost of fewer output spikes. From the perspective of downstream neurons that listen to mitral and tufted cells, excessive inhibition may generate distinctive but also very noisy patterns. At the limit, powerful lateral dendrodendritic inhibition might suppress all mitral and tufted cell responses except those driven by extremely active olfactory receptor cells, resulting in output patterns that are less distinctive than produced with minimal local circuit processing.

Inhibition is strongly modulated and occurs in mitral cells at different latencies during sensory responses (Hamilton and Kauer, 1989). Applying similar molecular approaches may reveal whether the dynamic nature of mitral cell inhibition reflects activity in excitatory cortical

feedback projections to granule cells, as predicted by in vitro electrophysiological recordings (Balu et al., 2007). The next step in understanding how bulbar interneurons function will be to follow the activity of populations of granule cells during olfactory behaviors. This is already possible in zebrafish, where Yaksi and Friedrich (2006) have discovered important differences between sensory representations in mitral and granule cell populations. We know now that a specific type of olfactory bulb interneuron plays an important role in olfactory discrimination. Hopefully this discovery will lead more researchers to push their electrodes, and their microscope objectives, beyond the mitral cell layer and into the realm of the interneuron.

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